Reappraising Starling: the physiology of the microcirculation

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Purpose of review
Vascular permeability is traditionally explained by Starling's principle, describing two opposing forces across the endothelial cell line to maintain compartments in balance. Several contradictions to this principle have recently questioned its validity.

Recent findings
Hydraulic conductivity is kept low by a properly working endothelial surface layer, created by binding and intercalating plasma constituents with the structural elements of an endothelial glycocalyx. Limiting fluid filtration is not closely related to the interstitial protein concentration. Rather, the oncotic pressure difference pertinent to fluid homeostasis is built up between the intravascular space and a small protein-free zone beneath the protein-loaded endothelial glycocalyx. This crucial structure, and therefore the resistance of the barrier against outflow of large molecules, is endangered by ischaemia, inflammation and intravascular hypervolaemia. An intact endothelial surface layer retains iso-oncotic preparations of large molecules infused to compensate for acute bleeding. Crystalloids cannot be held back sufficiently, even if preload is warranted.

Summary
Starling's principle requires an adaptation to recognize that there is no inward-directed oncotic pressure gradient across the whole anatomical vessel wall. The carrier of vascular barrier competence is the intact endothelial surface layer which might be protected by avoiding intravascular hypervolaemia and limiting inflammation.

Keywords
endothelial glycocalyx, endothelial surface layer, endothelium, vascular permeability

INTRODUCTION

The target of circulatory therapy is the microcirculation. Unfortunately, despite promising efforts [1▪], it still cannot be reliably assessed in clinical routine. This frequently leads to disregarding it in clinical practice, focusing treatment on cardio-circulatory surrogates. However, the 'stabilized' critically ill patient often bares surprises of what actually happens below the surface [2]. Despite the fact that appropriate surrogates combined to a pragmatic approach can be successful, some patients might benefit from a better understanding of the physiology of compartments and barriers.

PROPAEDEUTICS OF HUMAN PHYSIOLOGY

Protozoa consist merely of one compartment which is directly in touch with the environment; they do not need a circulation. During evolution, single cells were organized to organs and higher organisms. This is a problem for the nutrition of parenchymal cells which are now sealed within a body, far away from the substrates. The formerly unlimited extracellular space is now dramatically reduced to a closed compartment ('interstitium'), being quickly overwhelmed by cell metabolism if not permanently disposed and supplied. Nature has assigned this latter mission to the circulation. Cardiac arrest impressively demonstrates what happens if this system fails. The anatomical basis, the intravascular compartment, is part of the extracellular space,

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Curr Opin Crit Care 2013, 19:282–289

DOI:10.1097/MCC.0b013e3283632d5e

KEY POINTS

- The endothelial glycocalyx covers the entire vascular lumen of healthy blood vessels and forms an endothelial surface layer by binding plasma constituents.
- The vascular barrier consists anatomically of the endothelial cell line and the endothelial surface layer.
- The carrier of physiological vascular barrier competence is the endothelial surface layer as the inward-directed oncotic pressure gradient is derived across the glycocalyx.
- If the vascular barrier is intact, iso-oncotic preparations have a volume effect far beyond that of crystalloids.
- Sepsis, ischaemia, diabetes, arteriosclerosis, trauma and hypervolaemia can deteriorate the vascular barrier.

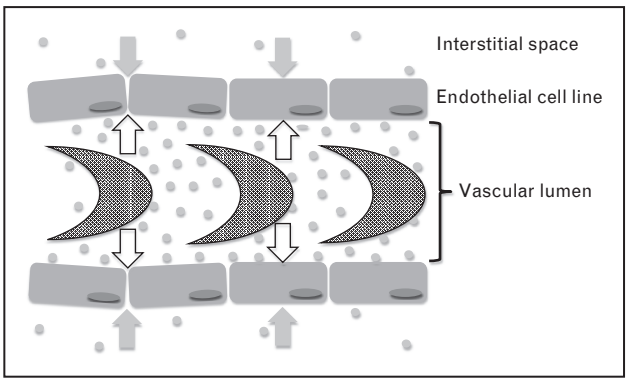

FIGURE 1. The vascular system is under (blood) pressure (white arrows), which is, according to the traditional view, opposed by the inward-directed oncotic gradient (grey arrows) generated across the vascular wall by a difference in the protein (grey circles) concentration.

functionally separated from the rest by the vascular barrier.

The water content of healthy, normal weight adults lies around 60% (45 l) under steady-state conditions. About 2/3 (30 l) is stored intracellularly, whereas 1/3 (15 l) forms the extracellular compartment. The latter consists by 80% (12 l) of the interstitial space and contributes by 20% (3 l) as plasma to cardiac preload. Water distribution between these two compartments is driven by hydrostatic pressures and the distribution of osmotically and oncotically active substances. Water by itself is not retained at any barrier within the human body.

External heart work permanently generates a longitudinal hydrostatic gradient from the heart via the arterial site across the microcirculation towards the large veins and back to the heart, sustaining blood flow. At the same time, this (blood) pressure permanently tries to force blood components across the vascular barrier towards the interstitial space, especially in the arterial system (Fig. 1). Under physiological conditions this is prevented by an intact vascular barrier.

ERNEST STARLING'S HISTORICAL CONCEPT: A HIGH FILTRATION–REABSORPTION SCENARIO

In 1896, Ernest Starling [3] published his model of microvascular fluid dynamics. On the basis of the available knowledge at this time, he suggested the endothelial cell line to resist the hydrostatically driven outflow of plasma constituents. Additionally, Starling presumed a protein-poor interstitial space, schematically drafting an oncotic gradient back towards the protein-rich plasma, binding water intravascularly and opposing the outwards directed force.

According to Ernest Starling [3], filtration behaviour across the vascular barrier of a non-fenestrated microvessel is traditionally described by the following equation:

$$F = \mathrm{HC} \times [(PV - PI) - (\pi V - \pi I)]$$

where F is the filtration rate per unit area per time; HC the hydraulic conductivity of the vascular barrier (amount per pressure per time); $\mathrm{PV} - \mathrm{PI}$ the hydrostatic pressure P difference between the vascular lumen V and the interstitial space I; $\pi V - \pi I$ is the oncotic pressure π difference between the vascular lumen and the interstitial space.

The story of Ernest Starling, ignoring the dispensable reflection coefficient for proteins, is that of two competing pressure gradients and the physical resistance of the vascular barrier against water passage. In this theory, the type of protein or colloid which is active at the endothelial surface is irrelevant, it exclusively calculates with colloid osmotic pressure (COP). 'Hydraulic conductivity', represented by the angle alpha, is an intrinsic property of the barrier itself and has nothing to do with additional forces. Principally, an increasing hydrostatic pressure gradient ($PV - PI$; X-axis) across a biological membrane leads to an increase in the filtration rate (Y-axis), the intersection lying in the point of origin (Fig. 2, black graph) [4].

Additionally, considering Starling's suggestion of an opposing inward-directed oncotic force across the whole anatomical vessel wall does not change hydraulic conductivity (graph steepness), but moves the graph rightwards (Fig. 2, grey graph). The new

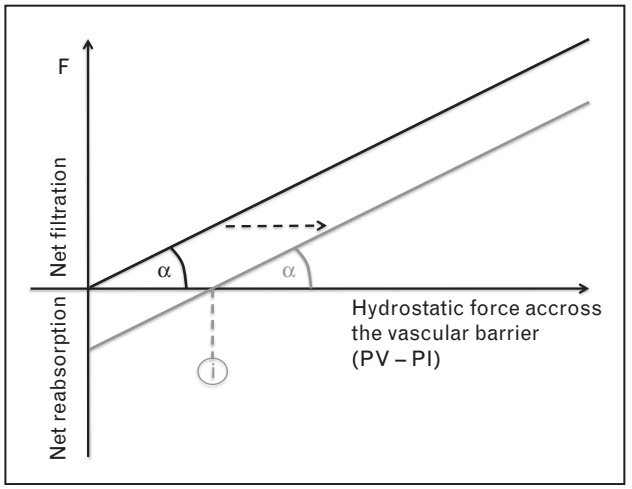

FIGURE 2. Net filtration behaviour across a non-fenestrated biological barrier when considering only a hydrostatic gradient (black graph) or with an additional inward directed force (grey graph). *F*, filtration rate per unit area; intersection *i*, net force opposed to the outward-directed hydrostatic gradient; *PV – PI*, hydrostatic pressure difference between the vascular lumen and the interstitial space (adapted with permission from [4]).

intersection '*i*' with the abscissa represents the net force opposed to the outward-directed hydrostatic gradient. The outward-directed force has to exceed the inward-directed force to start fluid filtration. Beyond that point, Δfiltration rate per Δhydrostatic pressure is the same as without. However, if the intravascular hydrostatic pressure falls below '*i*' – a condition which Ernest Starling [3] expected for the venular aspect – then fluid should be reabsorbed back into the intravascular space, which is an important part of this historical principle.

Following Starling's theoretical considerations, the two competing forces at the vascular wall might result in net filtration of water and small molecules towards the interstitial space in the high-pressure arteriolar segment. In the venular aspects, the inward-directed force should exceed the locally low intravascular pressure and most of the filtered volume is reabsorbed, obviously after a kind of fluid bypass across the interstitial space. Lymphatic drainage of any excess should keep the tissues in balance (Fig. 3) [5].

Increasing evidence from the past decade, however, revealed several contradictions to this historical model, indicating that it might require an update [6▪].

THE FIRST CONTRADICTION TO STARLINGS PRINCIPLE

One of Starling's basic presumptions is a protein-low area beyond the vascular barrier. This presumption does not reflect reality; the interstitial space is packed with albumin [7]. Already in 1991, Levick [8] formulated his 'low lymph flow paradox'. It was based on observations that most tissues would not be in balance if the inward-directed oncotic gradient across the vascular wall would be the only force limiting net pressure-dependent fluid filtration [9]. Adamson *et al.* [4] provided a quantitative idea of what is happening: in an isolated microvessel

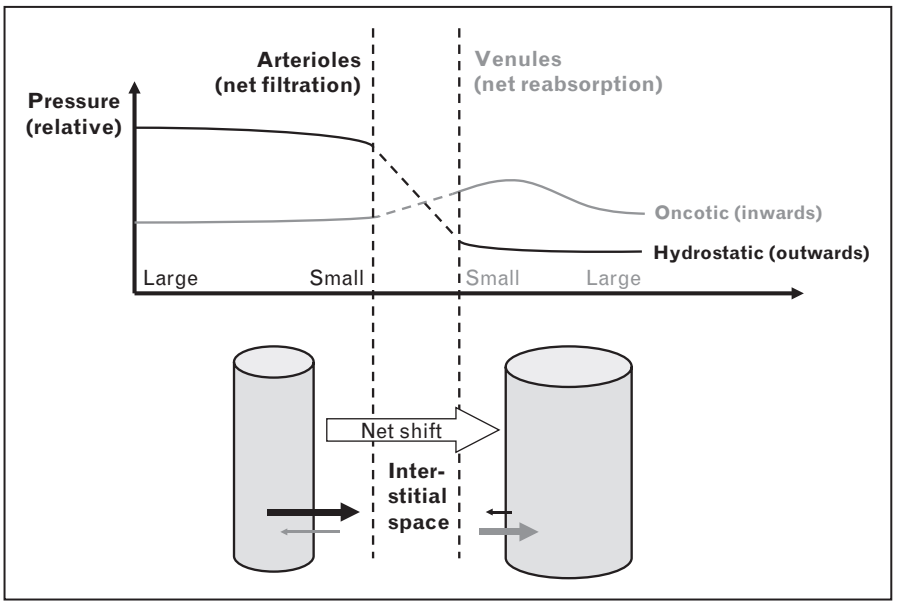

FIGURE 3. The filtration behaviour presumed by Ernest Starling in different sections of the vasculature. Black line: hydrostatic pressure gradient. Grey line: colloid osmotic pressure gradient (adapted with permission from [5]).

model they modified the oncotic pressure within the medium around, their model 'interstitial space'. They observed that a protein-free interstitial space – as suggested by Ernest Starling – was related to the expected pressure-dependent filtration behaviour across the vascular wall (cf. Fig. 2, grey graph). Obviously, an inward-directed force limiting pressure-dependent fluid filtration was active. Surprisingly, adapting the oncotic pressure of the artificial interstitium to the intravascular pressure did not relevantly change the conditions: the remaining intersection with the abscissa ('*i*') indicates a force opposing the intravascular pressure and limiting the model lymph flow. Obviously, nature does not fit into the historical equation of Ernest Starling, suggesting unlimited pressure-dependent fluid filtration for a failure of $(\pi V - \pi I)$ [3]. Rather, independently of πI, the barrier works.

The force limiting pressure-dependent fluid filtration is independent of the interstitial protein concentration.

THE SECOND CONTRADICTION TO STARLING'S PRINCIPLE

When describing the mechanism of action of intravascular proteins and colloids, traditionally only oncotic gradients are considered. This sight appears to be incomplete; the interaction of intravascular macromolecules with the endothelial surface is much more complex. In the coronary system of isolated guinea pig hearts, perfusion with a colloid-free perfusate led to high transvascular fluid shifts [10]. As expected, adding artificial colloids enhanced vascular barrier competence. This effect, however, was clearly inferior to perfusion even with low concentrations of human albumin. The graph representing pressure-dependent net fluid filtration was not primarily shifted towards the right. Remarkably, and quantitatively much more important, albumin in sub-physiological concentrations (1 g%, 1/4 of the physiological concentration) lowered hydraulic conductivity, flattening the curve (Fig. 4). This observation was independent of the intravascular existing COP, an effect termed the 'COP paradox' [7].

The intravascular presence of a low concentration of albumin does not primarily add another force to the system but gears into the central barrier immanent property 'hydraulic conductivity'.

THE THIRD CONTRADICTION TO STARLING'S PRINCIPLE

Already in 1653, Olaus Rudbeck showed that lymphatic fluid contains all clotting factors and is able to coagulate [11]. Meanwhile, it has been

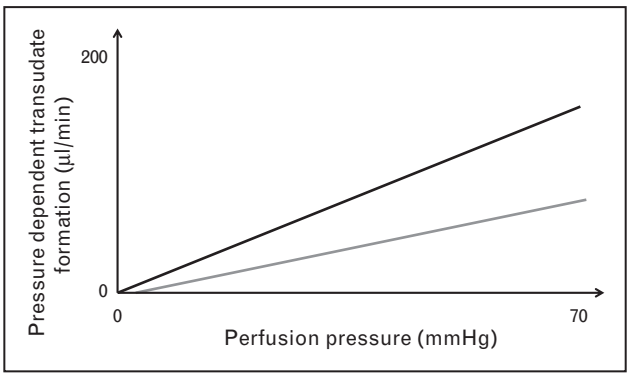

FIGURE 4. Pressure dependence of transudate formation in guinea pig hearts when perfusing the coronary system with a protein-free Krebs–Henseleit buffer (black graph) or when adding sub-physiological doses of human albumin (1 g%, grey graph) (published with permission from [10]).

demonstrated that tissue factor, a potent initiator of coagulation, is highly concentrated around high pressure segments of the microcirculation [12–14]. The biological intention is most likely the formation of a 'haemostatic envelope', immediately sealing the arterioles in case of injury [15]. Under physiological conditions, tissue factor is sufficiently separated from the flowing blood [16]. On the one hand, an excellent defensive strategy, on the other contradicting Starling's view of arterio-venular shifting, as a bypass of coagulation factors would plug the interstitial compartment with fibrin. Meanwhile, it has been confirmed that a fluid transfer across the interstitial space under physiological conditions is unlikely [4]: fluid is not reabsorbed at the venular aspect, but filtered at a variable extent throughout the vasculature. Fluid excesses are returned exclusively via the lymphatic system back into the circulation [9].

An aterio-venous fluid bypass across the tissues does not comply with the available data.

THE VASCULAR BARRIER: AN UPDATE

The past decade brought astonishing new insights into the biology of the vascular barrier, beyond the well known existence of the endothelial cell line. Obviously, the whole endothelial surface is covered by a glycocalyx [5,17–20]. In 1966, first electronmicroscopical pictures appeared, but an insufficient fixation technique led to its underestimation in size and importance [21]. Fifteen years later, a 'fibre-matrix model of capillary permeability' [22] was established before Pries *et al.* [19] introduced their ideas about an 'endothelial surface layer', presumably carrying most of the vascular barrier competence. Modern fixation techniques for electron

microscopy revealed a true thickness of the endothelial glycocalyx of up to 1 μm [23]. Beyond vascular barrier functioning, other important properties like anti-inflammation [2] and shear-stress transduction to the endothelial surface [24] have been attributed to this structure.

The endothelial glycocalyx is composed of membrane-bound glycoproteins and proteoglycans, mainly syndecan and glypican, carrying negatively charged side chains (mainly heparan, but also dermatan and chondroitin sulphates) and hyaluronan [5,19,20]. The glycocalyx itself, however, is nothing more than a skeleton. *In vivo*, by binding plasma constituents (mainly albumin), it is completed to the endothelial surface layer [5,19]. Only this entire structure is biologically active as a vascular barrier deserving this denomination, integrating the minimal possible hydraulic conductivity [10] and an adequate capacity to hold back large plasma molecules, providing a filtration-limiting inward-directed oncotic force. The site of this gradient appears to lie completely on the luminal side of the anatomical vessel wall [4,7].

THE VASCULAR BARRIER WITHIN THE MICROCIRCULATION: A COMPLEX AND FRAGILE SYSTEM

The available evidence commends that filtration behaviour in arterioles and capillaries is principally different from that across the wall of venules (Fig. 5). The endothelial surface layer limits filtration mainly within the high-pressure segments: hydrostatically outward-driven plasma components are retained here at the intact glycocalyx, increasingly loading it with COP [7]. Therefore, the glycocalyx acts like a sponge, separating large proteins from the ultrafiltrate. This generates a 'protected region' of some tens of nanometres with a very low protein concentration directly beneath the glycocalyx, but completely on the luminal side of the endothelial cells [5]. The latter are connected by tight junctions, principally not allowing water passage but being repeatedly interrupted by discontinuities within the junction stand [4], conducting the protein-poor ultrafiltrate towards the interstitial space. This permanently cleans the 'protected region' and prevents a back diffusion of proteins towards this sub-glyceal space, maintaining the inward-directed gradient [25]. Therefore, the intravascular COP only shifts pressure-dependent filtration behaviour to the right (cf. Fig. 2) within the high-pressure segment and on the basis of an intact endothelial surface layer, additionally keeping hydraulic conductivity low [7]. According to experimental evidence, the latter condition should be given even at plasma albumin concentrations of 1 g%. However, there are no clinical data evaluating such a threshold in human patients.

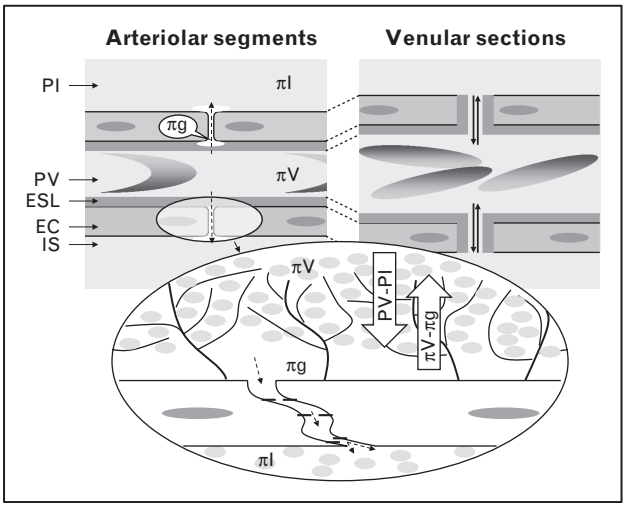

FIGURE 5. The revised model of vascular barrier functioning. Within the high-pressure segments ($PV \gg PI$) a tight endothelial surface layer guarantees a low hydraulic conductivity and the low oncotic pressure directly beneath the layer counts as opposed to that within the distribution space of the blood cells ($\pi V \gg \pi g$; left-hand panel), the resulting net outflow (small dotted arrows) being minimal. At the venular segment, by contrast, hydraulic conductivity is high, but due to the locally low hydrostatic pressure, diffusion into both directions (small black arrows) but not much net outflow results (right hand panel) adapted with permission from [5,7]. EC, endothelial cell line; ESL, endothelial surface layer; IS, interstitial space; *P*, hydrostatic pressure; π, oncotic pressure; *I*, within the interstitial space; *V*, within the vascular lumen; *g*, within the protected region below the ESL.

Within the venular aspect, the system might be much more penetrable for large molecules. As the hydrostatic force here is low, this should not cause a quantitative problem. Despite the fact that no venular net reabsorption occurs, extravasation of hormones, vitamins, lipoproteins and so on to supply the tissues is no problem, including back diffusion towards the vasculature [7]. Fluid filtered from any part of the circulatory system can be brought back towards the circulation via the lymphatic system. If its capacity is exceeded, interstitial oedema occurs.

Unfortunately, many patho-physiological situations in the perioperative and ICU setting are able to deteriorate this fragile structure. Sepsis, ischaemia/reperfusion, diabetes, trauma, resuscitation and arteriosclerosis have been shown to have a dramatic impact on the integrity of the glycocalyx [26–30]. Several inflammatory mediators have been shown

to compromise the glycocalyx [31–33]. But also surgical trauma, inflammation and iatrogenic intravascular hypervolaemia endanger vascular barrier competence [34].

FLUID PHYSIOLOGY MEETS CLINICAL PRACTICE: THE TRUTH ABOUT VOLUME EFFECTS

Recent clinical evidence around the efficacy of infusion measures fits impressively into these new physiological insights. In principle, two different model situations are of interest when intravenously applying i.v. fluids: infusion to maintain or restore blood volume in the context of acute bleeding, that is, when the system is in need of volume (clinical model: acute normovolaemic haemodilution – ANH [35]) and infusion in order to expand blood volume beyond intravascular normovolaemia, that is, when the system is not in need of volume (clinical model: hypervolaemic haemodilution [36]). Whereas the clinical impression including changes in haematocrit is occasionally misleading [37], only the combination of haemodilutional measures with direct assessment of the blood volume before and after can determine the intravascular volume effect. This was done prospectively for ANH with lactated Ringer and iso-oncotic preparations of human albumin and hydroxyethyl starch (Table 1) [35,38,39▪▪,40]. As expected, the crystalloid showed an intravascular persistence of around 20% [39▪▪].

The physiological reason should be primarily a dilution of the intravascular oncotic pressure (πV), increasing filtration mainly at the arteriolar site by shifting the pressure-dependent fluid filtration to the left. Perhaps a transient increase of the intravascular hydrostatic pressure (PV) affecting the whole system might also play a role here. Therefore, Starling was quantitatively absolutely right: the target compartment of an isotonic crystalloid is the extracellular space. Also a hypovolaemic circulation requiring restoration of cardiac preload is not able to retain oncotic pressure-free fluid. This 'type I shift' [34] is the predictable result of a chosen therapeutical concept and not associated with an alteration of the vascular barrier.

All tested iso-oncotic solutions, by contrast, remained almost completely within the circulatory compartment if infused to replace losses by acute bleeding [35].

Obviously, by more or less maintaining preexisting pressures around an intact vascular barrier by infusing iso-oncotic preparations during acute bleeding no relevant shift is induced.

Colloidal intravascular hypervolaemia, by contrast, led to a reduced intravascular volume effect of around 40% of the infused amount, 60% loaded the interstitial compartment as oedema [36]. This application of iso-oncotic colloids outside a proper indication significantly reduced the total volume of the endothelial surface layer to 1/3 of the initial value, presumably representing a severe affection of vascular biology.

Intravascular hypervolaemia causes a release of ANP, inducing matrix metalloproteases [41] digesting the endothelial glycocalyx within a very short period of time. The observed marked 'type II shifting' [34] of protein-rich fluid towards the interstitial space is the clinical correlate of a significantly altered vascular barrier. This increases hydraulic conductivity and decreases the force opposing to the outflow of proteins by increasing the oncotic pressure below the endothelial glycocalyx (πg) – a severe pathophysiological problem that the patient has to deal with during the entire perioperative period.

REAPPRAISING STARLING: CONCLUSIONS FOR CLINICAL PRACTICE

Natural proteins do not merely provide intravascular colloid osmotic force, but are a vital part of the

Table 1. Directly measured volume effects

Model	Preparation	*n*	Blood volume before haemodilution (ml)	Blood withdrawal (ml)	Infused amount (ml)	Blood volume after haemodilution (ml)	Volume effect (%)	Reference
ANH	5% Human albumin	15	3.745 ± 313	1.150 ± 196	1.333 ± 204	3.482 ± 561	87 ± 14	[35]
		10	4.412 ± 588	1.576 ± 227	1.831 ± 271	4.376 ± 654	85 ± 16	[40]
	6% HES 130/0.4	10	4.142 ± 474	1.431 ± 388	1.686 ± 437	4.360 ± 1.083	98 ± 12	[38]
	6% HES 200/0.5	10	4.093 ± 491	1.269 ± 217	1.469 ± 246	4.150 ± 451	90 ± 18	[40]
	Lactated Ringer	10	3.959 ± 387	1.097 ± 285	1.343 ± 806	3.501 ± 499	17 ± 10	[39▪▪]
VL	5% Human albumin	10	4.189 ± 769	n/a	1.379 ± 128	4.713 ± 868	38 ± 21	[36]
	6% HES 200/0.5	10	4.215 ± 728	n/a	1.417 ± 209	4.818 ± 721	43 ± 26	[36]

Values are mean ± SD. ANH, actue normovolaemic haemodilution; HES, hydroxyethyl starch; *n*, number of patients; n/a, not applicable; VL, volume loading.

vascular barrier already at very low concentrations. This role can only insufficiently be taken over by artificial colloids. However, albumin plasma concentrations of 1/4 of the physiological value appear enough to make the system work. The rest of the required intravascular oncotic pressure to achieve plasma iso-oncoticity can, at least from a fluid-physiological standpoint, be provided by any macromolecule – the compartments will stay in balance. Infusing a crystalloid leads to a distribution by 80% of the infused amount towards the interstitial space under any condition. Avoidance of intravascular hypervolaemia promises to protect a significant part of the vascular barrier.

CONCLUSION

In every individual patient we should carefully consider whether the pathophysiological price of choosing crystalloids for substituting acute blood losses or to prevent or to treat shock is worth paying. On the contrary, systemic inflammation can have already severely altered the vascular barrier, considerably reducing the normally high intravascular volume effect of colloids. Only the treating physician is able to decide which aspect dominates in the individual patient.

Acknowledgements

None.

Conflicts of interest

The authors declare to have held lectures for Fresenius Kabi, B. Braun, Serumwerk Bernburg, Baxter, CSL Behring and Grifols. Also, there are scientific cooperations with Fresenius Kabi, B. Braun, CSL Behring and Grifols.

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